Table 1. R_F VALUES OF SYNTHETIC PEPTIDES RELATED TO PRISTINAMYCIN

Peptide	R_F^1	R_F^2	R_F^3
Pic–Thr	0·20	0·79	0·50
Pic–Thr–But	0·36	0·45	0·53
Pic–Thr–But–Pro	0·48	0·89	0·54
Pic–Thr–But–Pro–Damephe	0·40	0·61	0·60
Pic–Thr–But–Pro–Damephe–Pip–Phegly	0·34	0·74	0·59
Pic–Thr–O(Phegly)	0·30	0·50	0·62
Thr–But	0·53	0·15	0·18
Thr–But–Pro	0·15	0·22	0·26
But–Pro–Damephe	0·32	0·22	0·21
Damephe–Pip–Phegly	0·51	0·50	0·29
Pip–Phegly	0·33	0·09	0·17
Pic–Gly	0·75	0·41	0·38
Pic–Thr–Gly	0·48	0·55	0·46

The following abbreviations are used: Pic, 3-hydroxypicolinic acid; Thr, L threonine; But, D α-aminobutyric acid; Pro, L proline; Damephe, L para-dimethylamino-N-methylphenylalanine; Pip, 4-oxopipecolic acid; Phegly, L α-phenylglycine; Gly, glycine. R_F^1: R_F by thin-layer chromatography on silica gel with methanol. R_F^2: R_F by paper chromatography with butanol-acetic acid-water (7–1–2). R_F^3: R_F by paper chromatography with isoamyl alcohol-pyridine-water (35–35–30).

linear heptapeptide corresponding to the opening of the aforementioned cyclic lactone, and several oligopeptides, as indicated in Table 1, have been synthesized[12].

The search for the metabolites in urine was performed as follows:

(1) For the untransformed constituent I_A.

This investigation was carried out by thin-layer and paper chromatography as well as by biossay[12].

(2) For compounds arising from possible degradation of the *cyclo*peptide.

The search for the amino-acids was performed by chromatography of the urines by means of a Technicon automatic amino-acid analyser. Synthetic amino-acids and urine collected immediately before the ingestion of pristinamycin were used for reference purposes.

The assay of peptides which could occur from cleavage of the *cyclo*peptide, was carried out by two techniques: paper chromatography was used for comparison of urines with blanks containing synthetic peptides, and thin-layer chromatography on silica gel was used for a systematic search for peptides. Comparative chromatograms of the urines to be investigated, blanks, and urines with added pristinamycin, were developed. Parallel areas were cut out on each plate, silica gel corresponding to each area was collected and eluted and the eluate was hydrolysed. Quantitative assays of the hydrolysates were then performed in order to determine the presence or absence of peptides and to evaluate their qualitative composition.

(3) For modified hydrolysis products.

The results of the foregoing investigation suggested that a search be made for compounds the structure of which resulted from a modification of the metabolites derived from the *cyclo*peptide. Paper chromatography, especially on anion exchange paper, was used for this work.

A complete survey of the urines of volunteers, who ingested 4 g of pristinamycin over 32 h in four administrations, gave the following results:

(1) About 10 per cent of constituent I_A of pristinamycin was excreted without transformation.

(2) No single amino-acid derived from constituent I_A was excreted in any detectable amount in urine.

(3) No peptide metabolite could be identified by comparison with the synthetic peptides listed in Table 1. These include the linear heptapeptide which would result from opening the macrolactone and correspond to theoretical hydrolysis fragments of constituent I_A.

(4) However, some metabolites could be detected in small amounts; ultra-violet absorption and fluorescence showed that they were related to 3-hydroxypicolinic acid. The main product was identified as a derivative of 3-hydroxypicolinylglycine, but even this peptide disappears from the urine 6 h after the absorption of pristinamycin.

G. JOLLÈS
B. TERLAIN
J. P. THOMAS

Laboratoires de Recherches de la Société des Usines Chimiques Rhône-Poulenc, à Vitry-sur-Seine (Seine), France.

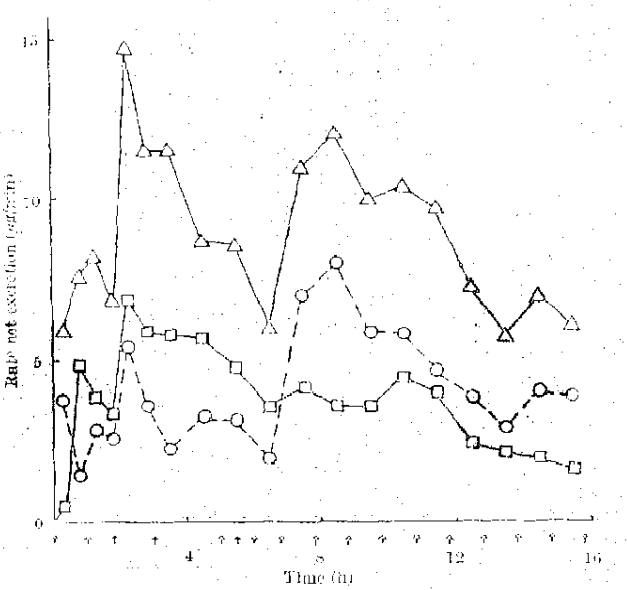
[1] Rhône-Poulenc, S. A., French patent 1,301,857 (June 27, 1961).
[2] Prud'homme, J., Bellec, A., Charpentier, Y., and Theridec, P., *C.R. Acad. Sci., Paris*, **260**, 1805 (1965).
[3] Barber, M., and Waterworth, P., *Brit. Med. J.*, 603 (1964).
[4] Bernard, P., Cesar, C., Duhost, M., Julou, L., and Marcy, D., *Semaine Thérapeutique*, **38**, 13 (1962).
[5] Colomb, D., and Pellerat, J., *Semaine Thérapeutique*, **38**, 71 (1962).
[6] Darbon, A., *Semaine Thérapeutique*, **38**, 45 (1962).
[7] Janbon, M., Brunel, D., Bertrand, A., and Michel-Briand, Y., *Semaine Thérapeutique*, **38**, 25 (1962).
[8] Mornier, J., and Tourse, R., *Semaine Thérapeutique*, **38**, 13 (1962).
[9] Sibouleh, A., *Semaine Thérapeutique*, **38**, 68 (1962).
[10] Eastwood, F., Spell, B., and Todd, A., *J. Chem. Soc.*, 2256 (1960).
[11] Jollès, G., Terlain, B., and Thomas, J. P. (to be published).
[12] Duhost, M., and Pascal, C. (to be published).

Significance of Smoking in Investigations of Urinary Excretion Rates of Amines in Man

IN the determination of amphetamine (T_R 9 min) by a gas–liquid chromatographic method[1], an additional peak (T_R 26 min) was observed in the extracts from urine of some but not all subjects; only subjects who smoked gave this latter peak. In smokers, large differences were observed in the 'apparent amphetamine' content of the urine determined by a methyl orange complexing method (using 1 : 2 dichloroethane as the extracting solvent, a solvent wash with borax *p*H 9·2 and measurements read at 540 mμ), and the amphetamine content determined by gas–liquid chromatography[1] (Fig. 1).

The additional component with a T_R of 26 min in the urine of smokers was shown to be nicotine by gas–liquid chromatography, thin-layer chromatography and also by the infra-red spectrum of the isolated base.

The sum of the amphetamine content and the nicotine content, both determined by gas–liquid chromatography, gave values comparable to the 'apparent amphetamine' content determined by the methyl orange technique.



Approximate times of smoking (each ↑ = 1 cigarette)

Fig. 1. True (□) and apparent (△) rates of amphetamine excretion in the urine of a smoker as determined by gas–liquid chromatography for the former, and by the methyl orange procedure for the latter. The rate of nicotine excretion (○) in the same urines is also indicated

The formation of complexes with methyl orange is a commonly used method for the determination of amines in urine and other biological fluids[2–8]. However, there seems to be little indication that only non-smokers have been used for investigations in man, although it has been reported[2] that nicotine complexes with the dye in the methyl orange technique. We have found that in many of the diverse methyl orange methods[4–8] the nicotine–methyl orange complex partitions into the organic phase and thus interferes with the determination of amines in biological fluids by these methods.

Using a gas–liquid chromatographic method for the determination of nicotine, we found that the rate of nicotine excretion in urine is influenced by the *p*H of the urine (see also ref. 9). Subjects smoking 20 cigarettes a day at fairly regular intervals excreted 0·1–4·3 (mean 1·0) μg nicotine per min under normal conditions, 0·4–13·0 (mean 5·0) μg nicotine per min when the urine was acidic after the oral administration of ammonium chloride, and less than 0·1 μg/min when the urine was alkaline after the oral administration of sodium bicarbonate.

Usually 'blank' values for urine by the methyl orange method are obtained before the method is used for the determination of drugs in urine. Obviously the 'blank' value for a smoker will depend on his smoking habits and the *p*H of the urine at the time of collection; this value may have little significance when applied as a correction at the time of the determination of the drug.

It is our opinion that smokers should not be used in urine excretion investigations of basic drugs unless nicotine has been shown not to interfere with the assay procedure adopted.

One of us (M. R.) thanks the Medical Research Council for a grant.

A. H. Beckett
M. Rowland
E. J. Triggs

School of Pharmacy,
Chelsea College of Science and Technology,
Manresa Road,
London, S.W.3.

[1] Beckett, A. H., and Rowland, M., *J. Pharm. Pharmacol.*, **17**, 59 (1965).
[2] Gettler, A. O., and Sunshine, I., *Anal. Chem.*, **23**, 779 (1951).
[3] Brodie, B. B., Udenfriend, S., and Dill, W., *J. Biol. Chem.*, **168**, 335 (1947).
[4] Chapman, D. G., Shenvey, K. G., and Campbell, J. A., *Canad. Med. Assoc. J.*, **81**, 470 (1959).
[5] Way, E. L., Swanson Rand, and Gamble, A. I., *J. Pharmacol.*, **91**, 178 (1947).
[6] Milne, M. D., Rowe, G. G., Semenek, K., Muschitzke, R. C., and Crawford, M. A., *Clin. Sci.*, **16**, 599 (1957).
[7] Lee, H. M., Scott, E. G., and Pohland, A., *J. Pharmacol.*, **125**, 14 (1959).
[8] Way, E. L., Sung, C. Y., and McKelway, W. P., *J. Pharmacol.*, **87**, 222 (1949).
[9] Haag, H. B., and Larson, P. S., *J. Pharmacol. Exp. Therap.*, **76**, 235 (1942).

Biological Half-lives of the Antibiotic Lincomycin Observed in Repetitive Experiments in the same Subjects

Some information on the disappearance half-time of lincomycin (the registered trade-mark of the Upjohn Company is 'Lincocin') has been reported by Ma, Lim and Nodine[1] and by Wagner and Alway[2].

We now wish to report biological half-lives of lincomycin estimated from serum levels obtained after oral administration of lincomycin hydrochloride in doses equivalent to 0·5 g lincomycin base. In three experiments (I, II and III) doses were administered to the same panel of six adult male subjects at 0, 7, 28, 35, 84 and 91 days. In experiment IV a panel of sixteen adult male subjects received doses one week apart. Table 1 lists the formulations and their code numbers used in the four experiments.

Experiments II, III and IV were cross-over studies in which half the subjects received one formulation and the other half received the other formulation the first week; the formulations being reversed with respect to subjects the next week. In each experiment blood samples were obtained at zero time (just before administration) and at 1·5, 3, 4·5, 6, 8, 10, 12 and 14 h after administration. Serum was collected and frozen. Antibiotic concentrations in serum were determined by the agar diffusion method with *Sarcina lutea* ATCC 9341 as the test organism. The method was the standard cylinder plate procedure of Henka *et al.*[3], as modified by Vavra *et al.*[4].

Table 1.

Expt.	Code	Type
I	1	Hard-filled capsule containing only lincomycin hydrochloride.
II	1	Fluid.
II	2	Aqueous solution formed by dissolving the above capsule contents in one-half glass of water.
III	3	Hard-filled capsule containing lincomycin hydrochloride, lactose, talc, and magnesium stearate.
III	4A	Paediatric solution containing lincomycin hydrochloride, preservatives, sweetening agents and flavouring agents.
IV	3	Fluid.
IV	4B	Paediatric solution — same formula as 4A but different lot.

Disappearance rate constants were calculated in the usual way[5] by first plotting the serum levels against time on semilogarithmic graph paper. All terminal points which appeared randomly distributed about a straight line were used. The method of least squares was employed using an IBM '1620' computer programme. The computer converted the serum levels to their logarithms (base 10) and printed out the slope of the least squares line. Division of the slope into 0·301 ($\log_{10}2$) gave the biological half-life, $t_{\frac{1}{2}}$.

The biological half-lives calculated from serum levels observed in repetitive studies in the same six subjects are shown in Table 2.

The biological half-lives calculated from serum levels observed in Experiment IV are shown in Table 3. The median half-life is 4·8 h, which agrees quite well with the overall median half-life of 4·6 h obtained in the other three experiments.

Analysis of the data shown in Tables 2 and 3 provides little, if any, evidence that the biological half life of lincomycin changes significantly with time when repetitive oral doses are given at weekly or less frequent intervals.

Ma, Lim and Nodine[1] reported a longer disappearance half-time of lincomycin after the last dose than that observed after the first dose when lincomycin hydrochloride was administered intramuscularly every 12 or 24 h to human subjects on a multiple-dose regimen. The difference in apparent average half-lives was not statistically significant, but they suggested that "further studies or the further analysis of available data should be carried out elucidating this point".

However, 'disappearance rates' and 'half times' reported by these authors following multiple doses were estimated from serum concentrations observed at 1 and 12 h or 1 and 24 h after administration. Our analysis of lincomycin serum levels following intramuscular administration indicates that part of the dose is absorbed over at least a 12 h period. Hence, Ma, Lim and Nodine estimated half-lives from data containing points in the absorption-tissue distribution phase, and not really a set of points randomly

Table 2. Biological Half-lives (in hours) of Lincomycin observed in Repetitive Studies in the Same Six Adult Male Subjects

Study	Date	Subject J	K	C	R	B	F	Date's Median
I	18/2/64	—	6·13*	—	5·59*	—	4·62*	5·36
	25/2/64	13·88*	—	5·87*	—	4·47*	—	5·67
II	24/3/64	4·69†	4·21†	4·43†	5·22‡	5·22‡	4·51†	4·47
	31/3/64	5·00†	5·02‡	4·58†	4·55‡	4·19†	4·55‡	4·68
III	19/5/64	7·85†	8·45§	5·15§	5·94‡	4·59§	4·47‡	5·37
	26/5/64	6·69§	4·30‡	4·22§	4·09§	4·18†	4·14§	4·20
Subject median		7·85	5·62	4·89	4·85	4·47	4·51	Median 4·80

* Formulation 1 (median half-life = 5·29 hours)
† Formulation 2 (median half-life = 4·58 hours)
‡ Formulation 3 (median half-life = 4·36 hours)
§ Formulation 4A (median half-life = 4·87 hours)